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Competition-colonization trade-off promotes coexistence of low-virulence viral strains

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Summary

RNA viruses exist as genetically diverse populations displaying a range of virulence degrees. The evolution of virulence in viral populations is, however, poorly understood. Based on the experimental observation of an RNA virus clone in cell culture diversifying into two subpopulations of different virulence, we study the dynamics of mutating virus populations with varying virulence. We introduce a competition-colonization trade-off into standard mathematical models of intra-host viral infection. Colonizers are fast spreading, virulent strains, whereas competitors are less virulent variants but more successful within coinfected cells. We observe two-steps dynamics of the population: Early in the infection the population is dominated by colonizers, which later are outcompeted by competitors. Our simulations suggest the existence of a steady state

in which all virulence classes coexist but are dominated by the most competitive ones. This equilibrium implies collective virulence attenuation in the population, in contrast to previous models predicting development of the population towards increased virulence.

Keywords: virus competition, virus dynamics, virulence, quasispecies, evolution

Introduction

The replication cycle of a particular viral strain can be described by different life history traits or fitness components, such as stability of viral particles, burst size, or virulence, among others [1, 2, 3, 4]. Variation of these traits affects viral fitness in different ways, and fitness components can be traded off against each other such that variation of one trait affects the other. Virulence is a phenotypic property of particular biomedical interest. In analogy with the virulence concept of epidemiology, we regard here the cytopathogenicity of the virus as its virulence. Accordingly, viruses with higher cell killing rate are considered to be more virulent.

RNA virus populations are exceptionally diverse due to the low fidelity of their replication process [5, 6]. The intra-host ensembles of strains, termed viral quasispecies, consist of mutant clouds of closely related but non-identical genomes [7]. The composition of a quasispecies is largely determined by the competitive fitness of its individual viruses [8]. Quasispecies diversity is the result of a balance between mutation and selection [9, 10]. The role of virulence in this intra-species competition is, however, unclear.

Several mathematical models have been designed to study the evolution of virulence under specific fitness trade-offs [11, 1, 2]. For example, the trade-off between virulence and transmission derives from the assumption that the longer a virus exploits its host, the higher the chances that it infects a new host [12, 13]. Under this assumption, it is predicted that if transmission is limited, virulence decreases and infections tend to attenuate over time [14].

However, the transmission-virulence trade-off, as postulated in epidemiological models, might not always operate in host-pathogen systems [15, 16]. Mutants of different RNA viruses, such as foot-and-mouth disease virus (FMDV) or Influenza, with a large difference in their cell killing capacity produce similar levels of progeny [17, 18, 19, 20]. Moreover, fitness and virulence are not necessary correlated traits [21, 22], thus suggesting that the trade-off between virulence and virus production does, in general, not hold at the cellular or intra-host level.

In a recent experiment with FMDV, two different phenotypes within the quasispecies were derived from a single purified clone. Each of these had adapted the ecological strategies of competition and colonization, respectively [19, 23]. Highly virulent viral strains play the role of colonizers, because they kill cells faster and thus replicate faster, which allows faster spread and colonization of new cells. Local competition arises when two or more different viruses infect the same cell and compete for intracellular resources. Competitors manage to produce more offspring in a cell coinfected together with a colonizer and, at the same time, extend the cell killing time characteristic of a colonizer, a phenomenon known as viral interference. A mixed competitor-colonizer population is subject to density-dependent selection. Under high density of viruses, competitors have an advantage because of the frequent occurrence of coinfections. Under low-density conditions, the virulent colonizers are selected because of their faster spreading through unoccupied cells. Density-dependent selection has been described for different RNA viruses [24, 25, 26, 27, 28], suggesting that competition and colonization might be general strategies of RNA viruses.

In the present study, we aim to understand how a competition-colonization trade-off shapes the evolution of virulence during intra-host infections of mutating viral populations. We employ suitably adapted deterministic models of virus population dynamics [29, 30, 31, 32], and model mutations using a transition matrix of probabilities between the different variants of the population defined by their virulence value. We compare the competition-colonization trade-off with the opposite assumption that more virulent variants are also more competitive, as previously suggested [33, 34, 35]. The major consequence of the competition-colonization trade-off is stable coexistence of multiple strains of reduced virulence that precludes a transient domination of virulent variants.

The model

The dynamics of intra-host viral infections have been studied using mathematical models [29, 30, 31, 32]. We make use of this well-established methodology while capturing the competition-colonization dynamics by representing multiple infections (i.e., coinfections) in the model.

Although in principle the same cell could be sequentially infected by a many strains, a virus infecting an already infected cell with sufficient delay after the initial infection will have a replicative disadvantage. The second strain would need to synthesize its own materials in a cell that may be partially or totally saturated . The delay in the superinfection would thus lead to a substantial competitive disadvantage. Accordingly, and to avoid a combinatorial explosion in the number of differential equations required, we limit the number of different virus types within coinfected cells to two, i.e., we consider only singly infected and doubly infected cells.

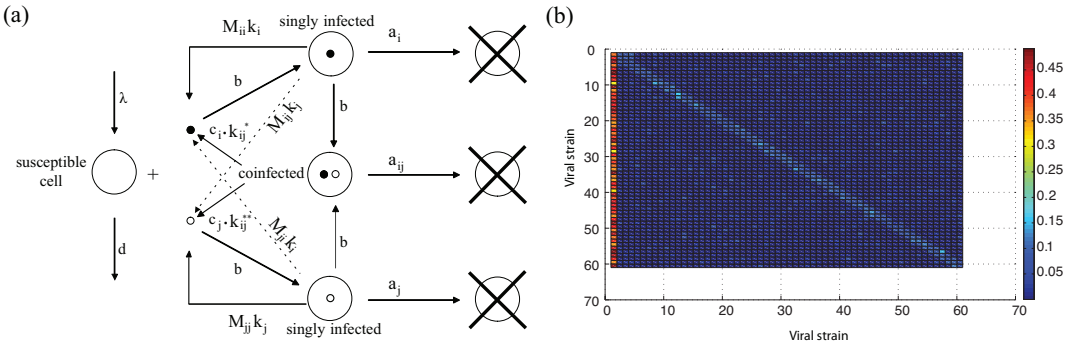

Figure 1: Schematic representation of the virus dynamics model. A cell pool replenished at constant rate λ becomes infected with efficiency β by colonizers, open circle, or competitors, filled circle, or by both in coinfected cells. Singly or multiply infected cells die and release viral offspring at rates a_i and a_{ij}, respectively. Free virus of type i is released by bursts of size K_i (or at rate $k_i = K_i/a_i$) and inactivated at rate u. Coinfected cells produce viruses of types $i = 1, \ldots, n$ at fractions proportional to c_i. M_{ij} is the mutation frequency by which strain j appears from strain i during the replication of strain i within a mono- or a co-infected cell. (*) The complete coefficients is $c_i k_{ij}(1 + M_{ji})$. (**)The complete coefficients is $c_j k_{ij}(1 + M_{ij})$. B) Transition probabilities between different strains of the population. The probability $M_{ij}$of the strain i on the first column to mutate and become the strain j of the first row is color coded according to the color bar

As shown in Figure 1a, we assume a renewed cell pool that can be infected by different viral strains. Competition between viral strains takes place at two different levels: viruses compete for the cell pool and inside coinfected cells. These dynamics are described by a multiple-strain SIR model defined by Equations(1) below and given in full generality in the Appendix.

We define M_{ij} as the relative frequency by which strain j arises from strain i due to mutations during the replication of strain i within a mono- or a co-infected cell. In order to assign realistic values to the entries of this matrix we explored the very scarce experimental literature investigating the effect of mutation on virulence. Concrete numbers could only be derived from the work of [22], in which viral strains are distinguished in terms of their fitness rather than their virulence. Nevertheless, based on the measurements obtained in [22], we chose the parameters of a Dirichlet distribution such that, on average, during the error prone replication of virus i within an infected cell 43% of mutations are lethal, 23% are neutral or lead to mutants with a virulence immediately close to the virulence of i, and 36% are mutants with a virulence more distant from i's 1b. The latter proportion is evenly distributed among all possible viable mutants with a virulence value not adjacent to i's. By sampling from this Dirichlet distribution we obtained the rows of the transition probabilities matrix depicted in 1b. We use this matrix as our model of mutations, which, with relatively high probability (on average 0.43) produces unviable mutants, favours transitions among close virulence values, and allows for sporadic jumps between far distant values. This matrix is kept constant for all simulations we performed in this study.

In sum, our model, including mutations, can be written as follows. For three viral strains, the model equations are as follows (See the Appendix for the model's equations in full generality):

$$
\begin{aligned}
\dot{x} &= \lambda - dx - \beta x(v_1 + v_2 + v_3) \qquad (1)\\
\dot{y}_1 &= \beta\left[xv_1 - y_1(v_2 + v_3)\right] - a_1 y_1\\
\dot{y}_2 &= \beta\left[xv_2 - y_2(v_1 + v_3)\right] - a_2 y_2\\
\dot{y}_3 &= \beta\left[xv_3 - y_3(v_1 + v_2)\right] - a_3 y_3\\
\dot{y}_{12} &= \beta(y_1 v_2 + y_2 v_1) - a_{12} y_{12}\\
\dot{y}_{13} &= \beta(y_1 v_3 + y_3 v_1) - a_{13} y_{13}\\
\dot{y}_{23} &= \beta(y_2 v_3 + y_3 v_2) - a_{23} y_{23}\\
\dot{v}_1 &= K\sum_{i=1}^{3}(M_{i1} a_i y_i) - uv_1\\
&\quad + K(M_{11}\left(c_{1,12}a_{12}y_{12} + c_{1,13}a_{13}y_{13}\right) + M_{21}\left(c_{2,12}a_{12}y_{12} + c_{2,23}a_{23}y_{23}\right)\\
&\quad + M_{31}\left(c_{3,13}a_{13}y_{13} + c_{3,23}a_{23}y_{23}\right))\\
\dot{v}_2 &= K\sum_{i=1}^{3}(M_{i2} a_i y_i) - uv_2\\
&\quad + K(M_{12}\left(c_{1,12}a_{12}y_{12} + c_{1,13}a_{13}y_{13}\right) + M_{22}\left(c_{2,12}a_{12}y_{12} + c_{2,23}a_{23}y_{23}\right)\\
&\quad + M_{32}\left(c_{3,13}a_{13}y_{13} + c_{3,23}a_{23}y_{23}\right))\\
\dot{v}_3 &= K\sum_{i=1}^{3}(M_{i3} a_i y_i) - uv_3\\
&\quad + K(M_{13}\left(c_{1,12}a_{12}y_{12} + c_{1,13}a_{13}y_{13}\right) + M_{23}\left(c_{2,12}a_{12}y_{12} + c_{2,23}a_{23}y_{23}\right)\\
&\quad + M_{33}\left(c_{3,13}a_{13}y_{13} + c_{3,23}a_{23}y_{23}\right))
\end{aligned}
$$

This ODE system describes the abundance of uninfected cells, x, that are replenished from an external supply at constant rate λ and die at rate d. Cells are infected by a variable pool of viruses v_i, characterized individually by the index according to their cell killing rate a_i. The infection takes place with efficiency β. Singly infected cells, y_i, and coinfected cells,

y_{jk}, die and release viral offspring at rate a_i and a_{jk}, the virulence of the respective strains. Free virus, v_i, is produced under mutation at rate M_{ji}, $j = 1, ..., n$, and lytic bursts of average size K. Free virus is inactivated at rate u. Typical values of the parameters, based on previous experiments with FMDV [36, 19] are $a_1 = 0.15\ h^{-1}$, $a_2 = 0.25\ h^{-1}$, $a_3 = 0.35\ h^{-1}$, $\beta = 5 \cdot 10^{-8}\ h^{-1}$, $K = 150$ viruses, $u = 0.15\ h^{-1}$, $d = 0.05\ h^{-1}$, and $\lambda = 10^5\ h^{-1}$.

The parameters $c_{i,jk}$ denote the proportion by which a cell coinfected with viruses of type j and k produce viral offspring of type i, where $i \in \{j, k\}$. We implement the competition-colonization trade-off by assuming intracellular competitiveness to be proportional to the reciprocal of virulence and set $c_{i,jk} = a_i^{-1}/(a_j^{-1} + a_k^{-1})$, and coinfected cells to die at the minimum rate of the two coinfecting strains, $a_{jk} = \min(a_j, a_k)$. For the alternative assumption of no intracellular viral interference, we set $c_{i,jk} = a_i/(a_j + a_k)$ and $a_{jk} = \max(a_j, a_k)$.

We investigate the n-viral-strains model (2) for a value of n large enough to model realistic populations with a broad spectrum of viral variants. The following initial conditions were used in all simulations: $x(0) = \lambda/d$, $y_i(0) = 0$, $y_{ij}(0) = 0$ for all $i, j = 1, \ldots, n$. The values of $v_i(0)$ are set according to suitable initial distributions of virulence further specified below.

Results

Competition-colonization dynamics. Based on the experimental data presented in [19], we have simulated viral coinfection dynamics using 60 different viral variants and their pairwise interactions under the competition-colonization trade-off $c_{i,jk} \propto 1/a_i$. According to this trade-off, the higher the virulence a_i of a virus, the lower the proportion $c_{i,jk}$ of the progeny produced in coinfected cells.

The range of virulence chosen was $[d, 0.5]$, where d is the natural death rate of uninfected cells. The upper bound of this interval is taken from the maximum cell killing rate described for FMDV, a highly pathogenic virus [36]. The choice of the lower bound d is based on the assumption that a viral infection significantly modifies the biology of the cell and increases

its death rate to the virulence of the infecting strain. Decreased cell death rates due to infection, as might occur with oncogenic viruses, are not considered here.

Every viral strain is defined by its virulence value and mutation is a frequency of transition between classes. The model is therefore conceived in such a way that all populations of - in terms of virulence - different mutants are modelled and mutations only give rise to such mutants. In other words, mutations do not generate new viral variants not contemplated a priori in the model .

This interval of virulence was equidistantly sampled yielding 60 different viable viral variants with a difference of virulence equal to $h := (0.5 - d)/60$ between adjacent strains. The lowest virulence value of d was assigned to all non-viable mutants that arise as a result of mutational processes. This assignment is not to be interpreted as the non-viable mutants having a very low virulence, because non-viable mutants are uncapable of infecting cells ($\beta = 0$) and thus the concept of virulence no longer applies. Rather, this assignment was made in order to preserve the structure of the implemented model for simulation purposes. The number 60 was chosen as a compromise to get sufficient coverage of the interval of virulence while keeping the computational cost of simulations in a reasonable range.

Competitor variants are the ones endowed with low a and high c, and colonizer variants the ones with high virulence a and low intracellular competitiveness c. According to the results of [19], the life span of coinfected cells cannot be statistically distinguished from the life span of cells singly infected with the least virulent strain. This observation suggested the use of the smallest a of the two coinfecting viruses as the per capita death rate of coinfected cells in our model.

The dynamics of this model are shown in Figure 2a. Uninfected cells become infected and produce progeny viruses during cell lysis. This process leads to a peak of viremia after about 10 to 20 hours. Afterwards, viremia slightly declines to an equilibrium value as a result of the balance between external supply of cells and virus-induced cell death. At early stages of the infection, virulent variants dominate the population. As the infection progresses, competitor

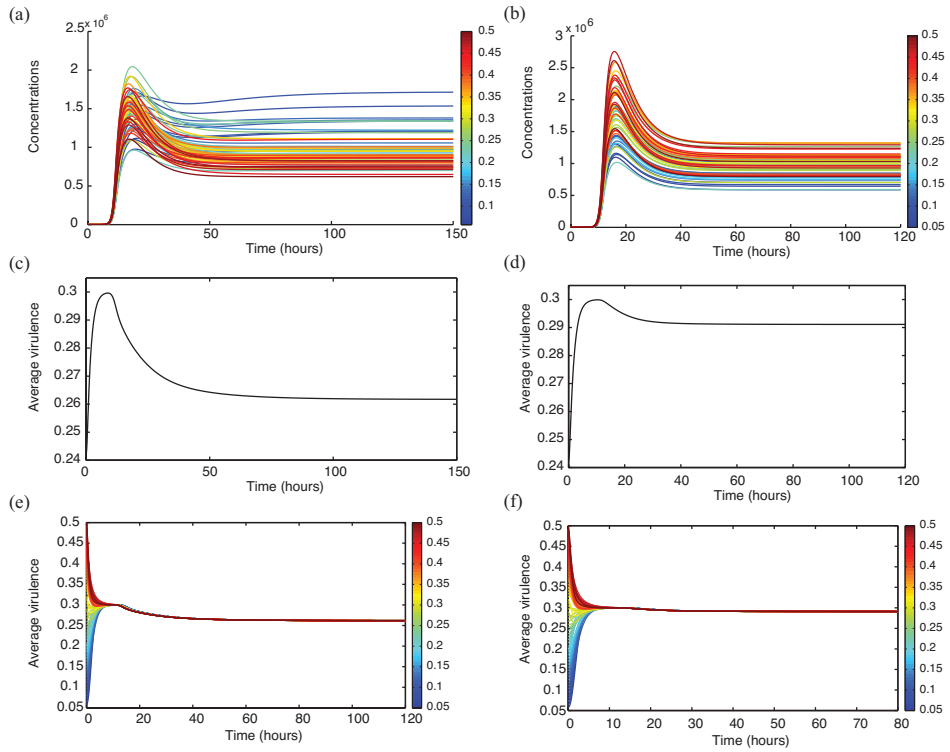

Figure 2: a) Time trajectories of the concentrations of 60 viral strains. Each curve displays the time evolution of the concentration of a different viral strain. Virulence values are color-coded according to the color bar. The initial concentrations are given by the initial multimodal distribution depicted in Figure 3. (a) Competition-colonization trade-off, described by $c_i \propto 1/a_i$. (b) Replication without interference, described by $c_i \propto a_i$. (c,d) Average virulence $\bar{a}(t)$ plotted over time during the corresponding infection shown in the above panel (d) Average virulence $\bar{a}(t)$ plotted over time during the corresponding infection shown in the above panel (e,f) Average virulence $\bar{a}(t)$ plotted over time corresponding to multiple infections starting with a single viral variant. The color code indicates the virulence of the initial strain. (e) Competition-colonization trade-off, described by $c_i \propto 1/a_i$. (f) Replication without interference, described by $c_i \propto a_i$.

variants (higher c) increase their relative abundances in the population. At equilibrium, competitors and colonizers coexist. The succession of competitors by colonizers eventually leads to attenuation, i.e., reduction of average virulence, of the whole viral population.

In order to assess the robustness of these findings with respect to variation of the model parameters and the initial conditions, we conducted many simulations with perturbed parameter values. The population size was fixed to 10000 viruses, and the proportion of each variant was randomly chosen in each simulation run. Among other instances, the simula-

154 tions included initial excess of either colonizers or competitors. Additional simulations were ran including an initial amount of viruses above the steady state viral load. All simulations
156 indicated that the equilibrium state, where the 60 variants coexist, remains invariant (data not shown), stressing the robustness of the model prediction regarding both, the qualitative
158 dynamics and the steady state.

Additional random variations of the remaining parameters affected only slightly the dy-
160 namics. The simulations were carried out using a Gaussian distribution of each parameter with mean equal to the typical value specified above and variance one half of the mean. Vari-
162 ations in burst size K, the external supply of cells λ, and the stability of viruses u, produced similar effects. The total viral load increased or decreased accordingly with variations of
164 the parameters, but the relative abundance of the strains at equilibrium remained constant. If β was varied, the dynamics run faster or slower, but the equilibrium was not affected.
166 Variations in the natural death rate of uninfected cells, d, had little or no effect at all on the dynamics or the equilibrium. When considering the higher cell death rate for coinfected cells
168 (under the current assumption the less virulent virus imposes its killing rate on coinfected cells) the dynamics of the infection progress faster, but the equilibrium abundance of viruses
170 is not affected.

In summary, the simulation results suggest that the model run with 60 viable and one pool
172 of unviable viruses has an asymptotically stable fixed point with a large basin of attraction.

Competition without intracellular interference. Many mathematical models for the
174 evolution of virulence in viruses do not take coinfections into account [14, 37, 12]. The amount of coinfected cells, however, has been proposed to vary linearly with the number of
176 singly infected cells [38].

When coinfections are considered, it is often assumed that parasites with higher virulence
178 outcompete less virulent strains also when coinfecting the same host, i.e., colonizers are also the better competitors [39, 34, 33]. This assumption is in contrast to our observations with

FMDV [19] and it neglects intracellular interference during replication in host cells coinfected with different variants [24, 25, 26, 27, 5, 40, 41].

For comparison with the competition-colonization assumption, we analysed the model of no intracellular interference by setting $c_{i,jk} = a_i/(a_j + a_k)$ and $a_{kj} = \max(a_k, a_j)$ in (2). The population dynamics of the two models are qualitatively different (Figure 2a,b). At early stages of infection, highly virulent strains have an advantage in both models. However, without intracellular interference, competitors never dominate in the population. The advantage of colonizers at the end of the infection is however slightly smaller than at the initial stages of the infection (see Discussion and Figure 2b).

Virulence evolution. The virulence of the whole population depends on the relative proportions of competitors and colonizers and their respective virulence levels. As a measure of population virulence, we consider the average virulence $\bar{a}(t) = \sum a_i v_i(t) / \sum v_i(t)$. We have analysed the time course of the population virulence for the two models discussed above (Figure 2c,d).

Under the competition-colonization trade-off, at early stages of the infection when the viral load reaches a maximum, the average virulence is maximal and the population is dominated by colonizers. Afterwards, both viral load and the average virulence decrease substantially. This final attenuation of the population is due to the dominance of competitors.

In the absence of intracellular interference, the population virulence dynamics shows a less pronounced qualitative change (Figure 2e). After the initial increase of virulence, the average virulence barely drops and stays high during the entire infection. Colonizers are always the dominant species in this type of competition.

In order to assess the strength of the attenuation effect in the competition-colonization dynamics, we have performed multiple simulations starting with a clonal population consisting of a single viral strain. For each such simulation, we chose a different initial strain and perform different simulations until the whole virulence spectrum considered in the model is

206 covered 2e,f. The virulence of the initial variant defines the initial average virulence of the population. During the infection, the mutations broaden the virulence spectra, attenuating
208 initially highly virulent populations, or increasing the virulence of less virulent ones. In all cases, under the effect of the competition-colonization dynamics, the average virulence
210 reaches a lower value at equilibrium than the one achieved without intracellular interference.

Thus, two different phenomena modulate the average virulence in the simulations, namely
212 the competition process and the diversity resulting from erroneous replication. While the competition tends to favour the competitor variants and attenuate the phenotype of the
214 population, the mutation effect is conditional to the initial diversity of the population. The different trajectories of virulence shown on Fig 3 collapse in the same value of average
216 virulence at steady state, suggesting the presence of an absorbing state.

Evolution of virulence distributions. In order to investigate the time evolution of
218 virulence in a diverse viral quasispecies under the competition-colonization trade-off, we need to keep track of the distribution of virulence during an infection. This population level
220 perspective on virulence is not revealed by summary statistics or consensus measures, nor is it easily accessible from the time trajectories of Figure 2.

222 Figure 3a shows the time evolution of a uniform initial virulence distribution of 60 different viral strains (see also Video 1 in the electronic supplementary material). The other
224 parameter values are the same as in the virus population simulations (Figures 2 and 3b).

In this simulation, we can observe the key qualitative features of the process. The time
226 evolution displays a two-steps behaviour. During the initial phase, the more virulent strains are amplified and the virulence distribution is in favour of colonizers. Then a qualitative
228 change occurs and the distribution becomes more neutral, without a bias towards extreme virulence values. Finally, the distributions changes again to give advantage to less virulent
230 competitors. This distribution becomes stationary. Figure 3b shows the time evolution of a less idealized initial virulence distribution (see also Video 2 in the electronic supplementary

material).

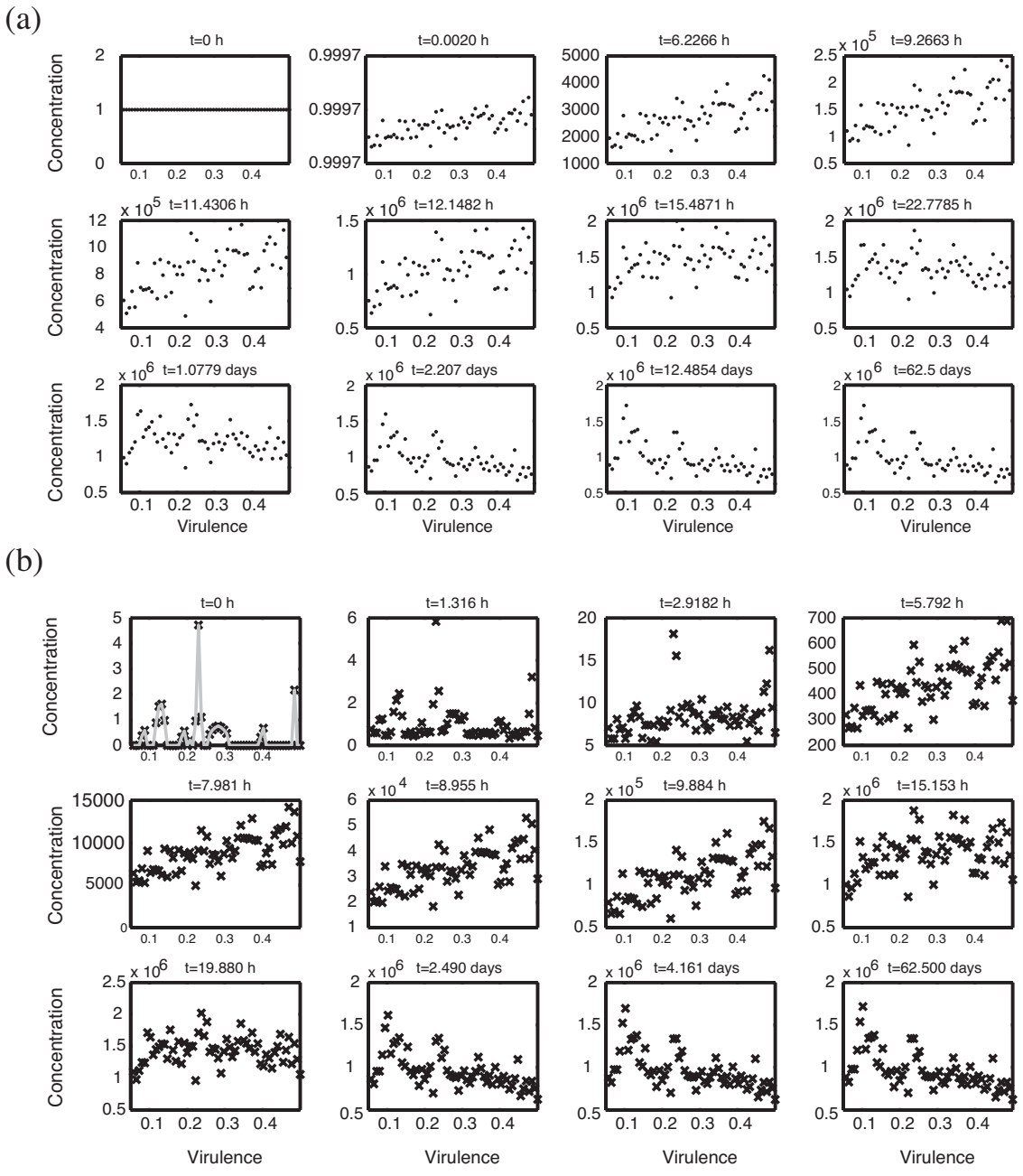

Figure 3: Virulence dynamics. (a) Time evolution of a uniform initial distribution of virulences. Each panel shows the distribution of virulence in the population (absolute concentration values for each virulence value) at the point in time displayed in its title. (b) Time evolution of a multimodal initial distribution of virulences.

The initial abundances of the 60 viral strains (Figure 3b, black crosses at $t = 0$) were obtained from a mixture distribution of seven Gaussian distributions with different means, variances, and weights (Figure 3b, solid line at $t = 0$). The time trajectory of this simulation is the one displayed in Figure 2. In this simulation, we again observe the two-step behaviour. A steady state is reached where all viruses coexist but competitors dominate.

Both simulations exemplify the mixing effect of mutations that eliminates the initial structure imposed by the initial distribution. Once the distribution has been mixed and enters a neutral phase (without a bias towards extreme virulence values), both simulations display similar dynamics and converge to the same stationary distribution. These results (and the results of other simulations not shown here) strongly suggest the presence of a globally attracting fixed point with a seemingly large basin of attraction.

Discussion

Experiments describing the molecular evolution of viral virulence are scarce and our knowledge about the mechanisms underlying this process is thus very limited. In a previous study, the diversification in cell culture of a clonal population into competitor and colonizer strategies was described in detail [19, 23]. In the present study, we adapted well-established mathematical models to assess the evolution of these two host exploitation strategies during intra-host infections.

We have assumed that two strategies are traded off against each other. The main difference between intra-host and cell culture infections is the presence of a replenished pool of susceptible cells *in vivo*. The constant supply of new cells gives continuity to the system and allows to assess the long-term behaviour of the population composition. We have simulated mutating, virulence-heterogeneous populations composed of 60 variants. All simulations predicted the same two basic features: sequential dominance of colonizers and competitors and the existence of a steady state of coexistence dominated by low-virulence competitors.

Competition and colonization strategies are subject to strong density-dependent selection [19, 23]. This type of selection can account for the observed sequential domination of the infection. Early in the infection, the density of viruses is very low due to the high availability of susceptible cells. The low density of viruses allows colonizers to spread faster in the initial stages of the infection. Progressively the density of viruses increases along with the number of coinfections. Since competitors are more efficient in intracellular replication, during later stages of the infection competitors take over and dominate in the population. The two-steps behaviour is maintained after a perturbation of the initial conditions. Even when competitors are initially dominant, they will be again replaced by colonizers .

Infections of the gypsy moth (*Lymantria dispar*) with nuclear polyhedrosis virus resembles the two-step dynamics described here [42]. During sequential passages (infections) of the virus from moth to moth, the virulence of the virus sampled at initial or later stages of the infection oscillates from high to low virulence, respectively. The lack of adaptive immune system, which is also not studied in our current model, may have contributed to the good fit of the results from both studies. Further experimentation along similar lines would be of great interest to understand the evolution of viral virulence in real infections, beyond cell culture experiments. A good test of our model would be to perform serial infections of animals with an RNA virus, taking samples of the virus for the next infection during the peak of viremia or at the steady state. Measuring the virulence of viruses obtained from each line of experiments would shed light on the evolution of virulence *in vivo*.

The switch of the favoured strategy, from colonization to competition, meets the replication requirements of the virus at each stage of the infection. Early in the infection the virus benefits from colonizing the organism as fast as possible, before the immune response is mounted. However, the less virulent variants have been predicted to maximize the viral load and the amount of infected cells. For a single virus model, if $R_0 \gg 1$, then the equilibrium abundance of viruses and infected cells is approximately given by $v^* \approx (\lambda k)/(au)$ and $y^* \approx \lambda/a$ [3]. These expressions imply that the equilibrium abundance of viruses and

infected cells will be higher in organisms infected by low virulence strains (low a). For this reason, once the organism is colonized, the viral population can benefit from the imposition of competitors.

The sequential replacement of colonizers by competitors during the infection of an organism has an interesting parallelism with ecological successions [43], were empty habitats are typically populated initially by fast spreading plants with shorter life cycles. Stronger competitors will successively replace the faster colonizers until the ecosystem reaches the climax.

Variability. The simulations suggest that the infection eventually reaches a steady state where all variants coexist. We have carried out a rigorous mathematical analysis of the two-virus model in the absence of mutations [44]. Under conditions that allow for viral spread (i.e., $R_0 > 1$), there is a local asymptotically stable equilibrium in which both viral strains coexist. The equilibrium abundances of viruses at the steady state satisfy $v_1^*/v_2^* = a_2/a_1$. This expression implies an advantage of strains of lower virulence in agreement with the observations derived from the simulations of the present work, although the effect is more moderate due to the smoothing effect of mutations.

In theoretical ecology a trade-off between the ability of each individual to colonize unoccupied territory and to compete with others for the same habitat patch has been suggested as a potential explanation for coexistence and species diversity in patchy habitats [33, 45, 46, 47, 48]. This trade-off has indeed been observed in plant and insects populations [49, 50, 51]. Our model is a space-implicit model, where the viruses replicate in patches defined by individual cells. The generation of new mutants by mutation and its fixation in the population are coupled in time in our error-prone model, unlike in classical ecology models. This fast dynamics prevents the complete extinction of any virulence class which can be beneficial when adapting to a different environment, for example, by increasing the invasive fitness when infecting a host that may be already colonized by another viral strain.

The variability, at a population-level, is a fundamental trait in the life cycle of RNA viruses, since they need to adapt to extremely changing environments. Pathology [52], fitness [53], evasion of antiviral drugs [54, 55], and immune response [6] are critically linked to the population diversity.

Virulence attenuation. Our simulations indicate that two mechanisms, mutation and the competition-colonization trade-off, modulate the composition of the population, sometimes in a complementary way, sometimes in an opposite way. Mutation tends to broaden the distribution of viral variants in the population. In those populations where the initial distribution of virulence values is highly skewed towards the domination of the more virulent variants, the low virulence variants will increase their proportion in the population simply by the effect of mutation. Conversely, initial virulence distributions highly skewed towards the presence of low-virulence competitors, they will see the amount of virulent colonizers increased over time. In addition to the impact of mutation on the steady state, the competition-colonization mechanism always favours the increment of the relative abundance of competitors at the steady state. If simulations are compared between populations with the same initial distributions of virulence values, but one subject to the rule $c_{i,jk} \propto 1/a_i$ (competition-colonization trade-off) and the other to $c_{i,jk} \propto a_i$ (replication without interference), the amount of competitors at equilibrium will be always higher under the influence of the trade-off. This results suggests an attenuating role for the competition-colonization mechanism in viral replication.

Attenuation has been documented for several infections, both at the intra-host level, and as a trend during epidemics [56, 57, 41, 58]. Our model has been derived from observations of real experiments carried out with different RNA viruses. The rationale for the trade-off between competition and colonization is that, during the replication of RNA viruses, negative-dominant mutants arise that can benefit from the replication of other mutants in coinfected cells. When coinfections occur, the population is enriched for these mutants called

competitors here, which act as defectors in the sense of evolutionary game theory [24]. Cell culture infections carried out at high density of viruses tend to select competitor strains that dominate over strains adapted to replicate without coinfections, as demonstrated for FMDV, vesicular stomatitis virus, and bacteriophage Φ6, among other viruses [24, 25, 59, 26, 27, 28]. In the extreme case, such defective mutants harbour internal deletions or lethal mutations and they require the coinfection of a helper virus to complete their replication cycle. It has been documented that defective viruses play a key role in the attenuation of several diseases [41]. This link between coinfection and disease attenuation is worth of further investigation as coinfections are frequent during virus-host infections [60, 61].

Despite the above-mentioned experimental evidence, the evolution of virulence has been classically studied under the contrary assumption of virulent strains being also more competitive. This assumption may hold for some parasites, such as bacteria or protozoa. These parasites do not necessarily exchange genetic products among individuals, which can result in limited interference [62, 63]. We have compared the competition-colonization trade-off with the situation where there is no interference between mutants, and the more virulent strain is also more efficient in coinfected cells. Our simulations suggest the existence of a steady state where different variants coexist dominated by virulent colonizers in agreement with previous work were the same assumption was done [34, 35]. Hence, this model would imply constantly increasing levels of virulence, in contrast to many experimental and clinical observations.

In conclusion, we have presented a model to study the evolution of virulence during virus-host interaction, which is based on experimental observations. Our results indicate that virulence is a dynamic feature of the entire population and the interaction between its components.

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Appendix: Mathematical Models

All models discussed in this paper are specializations of the following general multi-strain model

$$
\begin{aligned}
\dot{x} &= \lambda - dx - \beta x \sum_{k=1}^{n} v_k \\
\dot{y}_i &= \beta x v_i - \beta y_i \left(\sum_{\substack{k=1 \\ k \neq i}}^{n} v_k \right) - a_i y_i, \quad i = 1, ..., n \\
\dot{y}_{jk} &= \beta (y_j v_k + y_k v_j) - a_{jk} y_{jk}, \quad j, k = 1, ..., n \text{ and } j < k \\
\dot{v}_i &= K \sum_{k=1}^{n} (M_{ki} a_k y_k) + K \sum_{\ell=1}^{n} \left(M_{\ell i} \left(\sum_{\substack{j,k \\ j<k}} c_{\ell,jk} w_\ell(j,k) a_{jk} y_{jk} \right) \right) - u v_i, \quad i = 1, ..., n
\end{aligned}
\tag{2}
$$

where $w_\ell(j,k) = 1$ if $j = \ell$ or $k = \ell$, and otherwise $w_\ell(j,k) = 0$. The model does not explicitly account for the order of infection. The three-virus model (1) is a special case of this ODE system, obtained by setting $n = 3$. The competition-colonization model is derived from (2) by setting $a_{jk} = \min(a_j, a_k)$ and $c_{i,jk} = a_i^{-1}/(a_j^{-1} + a_k^{-1})$. The lack of intracellular interference is modelled by (2) with $a_{jk} = \max(a_j, a_k)$ and $c_{i,jk} = a_i/(a_j + a_k)$.

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